Development of Wheat Lines Having a Small Introgressed Segment Carrying Stem Rust Resistance Gene *Sr22*

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ABSTRACT

The wheat stem rust resistance gene *Sr22* confers resistance to *Puccinia graminis* f. sp. *tritici* Pers. race TTKSK (also known as Ug99) that developed in Africa and is an immediate threat to world wheat production. The resistance gene is present on a chromosomal translocation derived from *Triticum boeoticum* Boiss., which has a genome that is partially homologous to the A genome of *T. aestivum* L. *Sr22* has been deployed in a limited number of cultivars due to poor agronomic performance of lines carrying the resistance gene. Linkage analysis of simple sequence repeat (SSR) markers on chromosome 7A was performed to identify loci closely linked to *Sr22*. The most tightly linked proximal and distal SSR marker loci were *Xcfa2123* and *Xwmc633*, respectively. A two-step process was then used to develop resistant lines having smaller chromosome segments derived from the diploid donor. First, individuals in which a single recombination event had occurred between wheat and the *Sr22* introgression were identified in the mapping populations. In spite of reduced recombination between *T. boeoticum* and *T. aestivum* chromosomes, sufficient recombination events were found among 398 $F_{3:4}$ lines derived from recombinant F_2 progeny to recover multiple resistant individuals with smaller alien introgressions. Resistant lines were identified having less than 6% of the chromosome arm derived from *T. boeoticum.* These lines may provide a more agronomically desirable source of *Sr22* that can be readily deployed in cultivars resistant to Ug99.

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Abbreviations: FL, fraction length; IT, infection type; NIL, near isogenic line; PCR, polymerase chain reaction; SSR, simple sequence repeat.

Wild relatives of modern bread wheat (*Triticum aestivum* L.) are an important source of genes conferring resistance to disease and insect pests. Several genes for resistance to stem rust caused by *Puccinia graminis* f. sp. *tritici* Pers. (*Pgt*) have been introgressed from related species, including the adult plant resistance genes *Sr2* from the *T. turgidum* L. emmer wheat landrace 'Yaroslav' (McFadden, 1930), *Sr24* from tall wheatgrass [*Thinopyrum elongatum* (Host.) D.R. Dewey] (Sears, 1973), *Sr31* from rye (*Secale cereale* L.) (Zeller, 1973), and *Sr36* from timopheevi wheat [*T. timopheevii* (Zhuk.) Zhuk.] (Nyquist, 1962). These resistance genes have been widely deployed

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to provide stem rust resistance in many wheat growing areas of the world. Other broadly effective *Sr* genes transferred from wheat relatives, including *Sr32*, *Sr39*, and *Sr40*, remain undeployed in modern agriculture due to reduced agronomic performance associated with their presence on large alien chromosome introgressions (Singh et al., 2008).

The resistance gene *Sr22* was introgressed into common wheat from wild and cultivated A-genome diploid wheats (The, 1973). It provides effective resistance to the variant stem rust races of the TTKS lineage (also referred to as Ug99) (Jin et al., 2007) that developed in Africa and now threaten world wheat production. The *Sr22* gene was identified in the *T. boeoticum* Boiss. [syn. *T. monococcum* ssp. *aegilopoides* (Link) Thell.] accession G-21 (Gerechter-Amati et al., 1971) and the *T. monococcum* L. (syn. *T. monococcum* L. ssp. *monococcum*) accession RL5244 (Kerber and Dyck, 1973). Introgressions from the two donors differ in the amount of alien chromatin present. The *T. boeoticum* introgression into the common wheat cultivar Steinwedel includes nearly the entire long arm and a portion of the short arm of chromosome $7A^m$. The *T. monococcum* introgression into Marquis consists of the distal region of $7A^mL$ (Kerber and Dyck, 1973; Paull et al., 1994). Khan et al. (2005), using simple sequence repeat (SSR) markers, genetically mapped *Sr22* between *Xcfa2123* (proximal) and *Xcfa2019* (distal) with an interval distance of 11.2 cM, an interval previously estimated to be approximately 30 cM (Sourdille et al., 2005). The smaller genetic distance in the *Sr22* mapping population was presumably the result of reduced recombination between the introgressed segment carrying the gene and wheat chromosome 7A.

Reduced recombination between chromosomes of the A genome of *T. aestivum* and the A^m genome of einkorn wheat relatives has been well documented (Dubcovsky et al., 1995; Luo et al., 2000). The *Ph1* gene of hexaploid wheat restricts homoeologous pairing (Riley and Chapman, 1958), preventing recombination between homoeologous chromosomes. Consequently, introgressed chromosome segments from species that are not completely homologous may be inherited in large linkage blocks carrying substantial amounts of alien chromatin. Detriments to agronomic performance have been reported in association with the *Sr22* introgressions, including depression of yields and delayed heading date (Paull et al., 1994). In near isogenic lines (NILs) from different genetic backgrounds carrying *Sr22*, The et al. (1988) reported a mean yield that was not significantly lower than non-*Sr22* controls. However, the results varied among genotypes, with some *Sr22* NILs yielding as much as 10% less than non-*Sr22* controls. Paull et al. (1994) reported that lines without the *T. boeoticum* introgression headed approximately 6 d earlier than lines heterozygous and homozygous for the introgression. In addition, reduced transmission of gametes carrying *Sr22* has been observed for both the *T. boeoticum* and *T. monococcum* introgressions (The and McIntosh, 1975). Poor agronomic performance and reduced transmission of *Sr22* have been attributed to linkage drag resulting from substantial chromatin derived from the A^m genome relatives.

The associated negative effects on agronomic performance have hindered the use of *Sr22* worldwide. However, deployment of this gene is of particular interest in contemporary wheat breeding because it confers resistance to *Pgt* race TTKSK and more virulent derivatives that have emerged from East Africa (Singh et al., 2008). Additionally, *Sr22* confers resistance to all domestic *Pgt* races in the United States. In this study we report genetic linkage analysis of the *Sr22* region in multiple populations and the identification of more tightly linked SSR marker loci for use in marker-assisted selection. In addition, recombinants have been identified that carry reduced amounts of *T. boeoticum* chromatin that may prove useful in the deployment of *Sr22* in breeding populations.

MATERIALS AND METHODS

Plant Materials

For linkage analysis of *Sr22*, two mapping populations were developed. The resistant parent for both crosses was the germplasm Sr22Tb with the pedigree Steinwedel*2//Spelmar*2/*T. boeoticum* G-21. Seed of this Steinwedel selection that has the $7A^m$ chromosomal translocation from *T. boeoticum* carrying the stem rust resistance gene *Sr22* (The, 1973) was provided by Dr. Yue Jin, USDA-ARS Cereal Disease Lab, St. Paul, MN. Two Steinwedel accessions, PI 41081 and PI 27018, obtained from the USDA National Small Grains Collection were used to compare the haplotypes of SSR loci linked to *Sr22* between the germplasm Sr22Tb and the original cultivar Steinwedel.

A population of 138 $F_{2:3}$ lines, referred to as U5615-$F_{2:3}$, was developed from the cross of Sr22Tb with the hard winter wheat cultivar 2174 (PI 602595). An additional population of 139 $F_{2:3}$ lines, referred to as U5616-$F_{2:3}$, was developed from a cross between Sr22Tb and the hard winter wheat cultivar Lakin (PI 617032). From these populations, three $F_{2:3}$ families having recombination between distal marker *Xcfa2019* and proximal marker *Xbarc121* in the *Sr22* region were selected for development of additional mapping populations and identification of recombinants having smaller introgression segments. Linkage analysis was performed using SSR loci segregating in $F_{3:4}$ populations developed from the F_2 plants U5615-72, U5615-98, and U5616-20. From the recombinant plant U5615-72 that was homozygous for alleles from parent line 2174 at the distal *Xcfa2019* locus and heterozygous for *Sr22*, 104 $F_{3:4}$ lines were developed (population U5615-72-$F_{3:4}$). From the U5615-98 recombinant that is homozygous for alleles from parent line 2174 at the proximal *Xbarc121* locus and heterozygous for *Sr22*, 140 $F_{3:4}$ lines were developed (population U5615-98-$F_{3:4}$). A population of 152 $F_{3:4}$ lines was derived from the recombinant F_2 plant U5616-20 that was homozygous for Lakin alleles at the *Xbarc121* locus and heterozygous for *Sr22* (population U5616-20-$F_{3:4}$).

The chromosomal assignment of the SSR loci to the long arm of chromosome 7A was done using Chinese Spring nullisomic-tetrasomic and ditelosomic stocks (Sears, 1954; Sears and Sears, 1978) for this chromosome. Chromosome physical mapping of SSR loci was done using Chinese Spring chromosomal

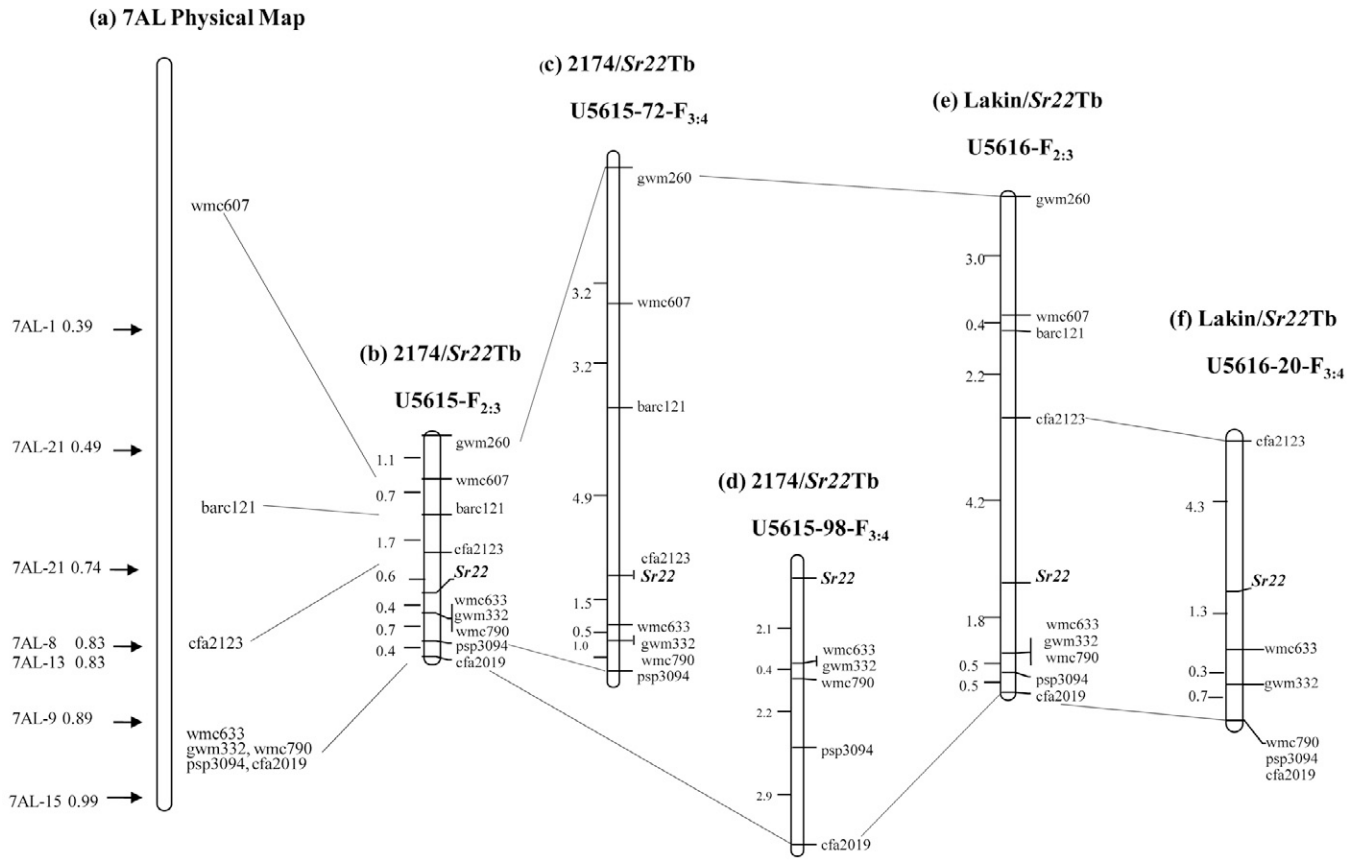

Figure 1. (a) Physical map of SSR loci linked to *Sr22* on the long arm of chromosome 7A (*Xgwm260* not shown because it is located on 7AS). Names of deletion lines and the deletion breakpoints shown at left. (b–d) Genetic linkage of $F_{2:3}$ population U5615 (2174/Sr22Tb) and $F_{3:4}$ populations U5615-72, U5615-98 derived from recombinant F_2 individuals segregating for stem rust resistance from *Sr22*. (e–f) $F_{2:3}$ population U5616 (Lakin/Sr22Tb) and $F_{3:4}$ population U5616-20 derived from a recombinant F_2 individual segregating for stem rust resistance from *Sr22*.

deletion stocks of 7AL. The lines used in deletion mapping include 7AL-15 (0.99), 7AL-9 (0.89), 7AL-13 (0.83), 7AL-8 (0.83), 7AL-21 (0.74), 7AL-10 (0.49), and 7AL-1 (0.39) (Endo and Gill, 1996) (Fig. 1a). All aneuploid and deletion stocks were obtained from the Wheat Genetics and Genomics Resource Center at Kansas State University, Manhattan, KS.

Stem Rust Evaluations

Puccinia graminins f. sp. *tritici*, race RKQQC (virulence/avirulence formula: *Sr5*, *6*, *7b*, *8a*, *9a*, *9b*, *9d*, *9 g*, *21*, *36*, *McN/Sr9e*, *10*, *11*, *13+17*, *24*, *30*, *31*, *38*, *Tmp* [based on revised nomenclature from Jin et al., 2008]) is avirulent to *Sr22* and was used for phenotypic analysis of mapping populations. At least 10 seedlings of all $F_{2:3}$ and $F_{3:4}$ lines and the resistant and susceptible parents were grown in 10 by 10 cm square pots in Metro-Mix 200 vermiculite–peat–perlite medium (Hummert, Inc., Earth City, MO) in a greenhouse. Urediniospores were removed from liquid nitrogen storage and heat-shocked in a 42°C water bath for 5 min. Spores were suspended in Soltrol 170 isoparaffin oil (Chevron Phillips Chemical Company LP, The Woodlands, TX) and sprayed onto two- to three-leaf stage seedlings. Inoculated plants were incubated in a dew chamber at 24 ± 1°C, 100% relative humidity for 16 h and then grown in a greenhouse at 21 ± 4°C with 16 h light/8 h dark cycle. Infection types (ITs) described by Stakman et al. (1962) were assessed 14 d after inoculation. Rust evaluations were replicated so that a total of at least 20 seedlings from each line were evaluated.

Stem rust reaction of parent lines and selected recombinants having reduced *T. boeoticum* chromatin were determined using eight races of *P. graminis* f. sp. *tritici*, including races of the TTKS lineage at USDA-ARS Cereal Disease Laboratory (St. Paul, MN). Inoculation and disease assessment procedures were reported previously (Jin et al., 2007). Virulence/avirulence formulas of the tested races are given in Table 1.

Molecular Marker Analyses

Genomic DNA was extracted from F_2 plants and from a bulk of 10 plants of each $F_{3:4}$ line. DNA isolations were done according to a protocol modified from Pallotta et al. (2003) or using a QIAGEN DNeasy 96 Plant kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

Parent lines 2174, Lakin, Sr22Tb, and Steinwedel were screened for polymorphism with 42 SSR markers previously mapped to the long arm of chromosome 7A including: barc29, barc49, barc108, barc121, barc174, barc192, barc195, barc221, cfa2019, cfa2040, cfa2123, cfa2257, cfd20, cfd68, cfd193, gwm4, gwm10, gwm63, gwm260, gwm276, gwm282, gwm332, gwm344, gwm473, gwm554, gwm573, psp3094, wmc17, wmc96, wmc116, wmc139, wmc107, wmc139 wmc273, wmc346, wmc426, wmc488, wmc525, wmc607, wmc633, wmc790, and

Table 1. Virulence and avirulence formulas for the *Puccinia graminis* f. sp. *tritici* races used in the phenotypic analysis of *Sr22*.

Pgt race†	Avirulence	Virulence
MCCFC (59KS19)	6 8a 9a 9b 9d 9e 11 21 24 30 31 36 38	5 7b 9g 10 17 Tmp McN
QFCSC (06ND76C)	6 7b 9b 9e 11 24 30 31 36 38 Tmp	5 8a 9a 9d 9g 10 17 21 McN
QTHJC (75ND717C)	7b 9a 9e 24 30 31 36 38 Tmp	5 6 8a 9b 9d 9g 10 11 17 21 McN
RKQQC (99KS76A)	9e 10 11 17 24 30 31 38 Tmp	5 6 7b 8a 9a 9b 9d 9g 21 36 McN
TPMKC (74MN1409)	6 9a 9b 24 30 31 38	5 7b 8a 9d 9e 9g 10 11 17 21 36 Tmp McN
TTTTF (01MN84A-1-2)	24 31	5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp
TTKSK (04KEN156/04)	24 36 Tmp	5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 31 38 McN
TTKST (06KEN19-V-3)	36 Tmp	5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 24 30 31 38 McN

†*Pgt* race code, after Roelfs and Martens (1988) using revised nomenclature from Jin et al. (2008). Race isolates given in parentheses.

wmc809. Markers amplifying polymorphic fragments were run on aneuploid and deletion stocks to validate their chromosomal location and then evaluated on mapping populations. Thirteen markers amplifying polymorphic fragments between Sr22Tb and both hard winter wheat parents and assigned to chromosome 7A were used for linkage analysis of segregating populations.

The polymerase chain reaction (PCR) master mix for SSR primers consisted of 2 μL of 20 ng μL^{-1} genomic DNA template, 0.40 μL of a 10 μM mixture of forward and reverse primers, 0.18 μL (0.9 U) of *Taq* polymerase, 1.20 μL of 10× buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3), 0.96 μL of a 100 μM mixture of dNTPs, and 7.26 μL of water, bringing the total reaction volume to 12 μL. A touchdown profile was used that consisted of an initial denaturation at 95°C followed by 15 cycles of 95°C (45 s), 65°C (45 s) decreasing by 1°C each cycle, and 72°C (60 s), followed by 25 cycles of 50°C annealing temperature. The cycling conditions for markers requiring specific annealing temperatures included an initial denaturation of 95°C followed by 35 cycles of 95°C (45 s), 49°C or 60°C (45 s), and 72°C (60 s), followed by a final extension at 72°C (4 min). The forward primers for all SSR markers were 5′-modified to include the fluorescent dye 6-FAM. Amplifications were performed using an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). Sizing of PCR products was performed by capillary electrophoresis using a 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA). Analysis of PCR fragments was performed using GeneMarker 1.60 software (SoftGenetics, State College, PA). Genetic linkage analysis was performed using MAPMAKER v 3.0 (Lander et al., 1987). Marker order was established using multipoint analysis and the Haldane centimorgan function with a minimum LOD of 3.0. Segregation of marker loci and resistance reaction was evaluated using a χ^2 goodness-of-fit test. Differences in recombination frequency between populations were determined according to Allard (1956).

RESULTS AND DISCUSSION

Phenotypic Evaluation

Germplasm Sr22Tb exhibited ITs of 2− or 2 when inoculated with diverse races of *Pgt*, including races TTKSK and TTKST (Table 2). Susceptible reactions (IT = 4) were observed on parent line 2174 when inoculated with six of the eight races of *Pgt*. Resistant reactions were observed when cultivar 2174 was inoculated with isolates MCCFC and RKQQC, indicating the presence of a race-specific stem rust resistance gene in this parent. The infection type was variable among different inoculation experiments when 2174 was challenged by RKQQC, suggesting the presence of an environmentally sensitive resistance gene(s) in this line (data not shown). The parent line Lakin was consistently susceptible to all isolates.

In each segregating population, individuals homozygous or heterozygous for *Sr22* exhibited a low IT of 1− to 2 on challenge with *Pgt* race RKQQC. Uredinia were typically small and surrounded by chlorosis rather than necrosis. Individuals in the populations with an IT of 2+ or greater were considered susceptible (*sr22sr22*).

Analysis of segregation ratios indicated that resistance to stem rust was due to a single dominant gene in both populations (Table 3). Although reduced transmission of *Sr22* had been previously reported by Paull et al. (1994), there was no significant deviation from the 1:2:1 segregation ratio expected for a single dominant gene. Encouragingly, the variable seedling resistance to RKQQC in 2174 did not interfere with our ability to score *Sr22* in F_3 families.

Genetic and Physical Mapping of the Sr22Tb Introgression

Of the 42 SSR loci evaluated, 13 were polymorphic between Sr22Tb and the hard winter wheat parents 2174 and Lakin. Linkage analysis of these makers on the U5615-$F_{2:3}$ and the U5616-$F_{2:3}$ populations placed the markers onto two linkage groups. Nine of the SSR markers were placed onto a linkage group with *Sr22*. These loci included *Xbarc121*, *Xcfa2019*, *Xcfa2123*, *Xgwm260*, *Xgwm332*, *Xpsp3094*, *Xwmc607*, *Xwmc633*, and *Xwmc790* (Fig. 1). Four other loci, *Xcfa2257*, *Xgwm344*, *Xwmc346*, and *Xwmc525*, formed a second unlinked group greater than 60 cM distal to *Sr22* (LOD > 3; map not shown). These four markers have previously been located terminally on 7AL (Miranda et al., 2007; Perugini et al., 2007).

The orders of markers in the *Sr22* linkage group agree in both populations (Fig. 1). The nine linked markers covered a region of 5.6 cM in the U5615-$F_{2:3}$ population, compared to a distance of 12.6 cM in the U5616-$F_{2:3}$ population. Thus, there was a twofold reduction in recombination in the 2174/Sr22Tb population compared with the population derived from the cross Lakin/Sr22Tb ($P < 0.01$). In

Table 2. Infection types of parent lines 2174, Lakin, Sr22Tb, and selected recombinants when inoculated with eight different races of *Puccinia graminis* f. sp. *tritici*.

Line	TTKSK (04KEN156/04)†	TTKST (06KEN19-V-3)	TPMKC (74MN1409)	TTTTF (01MN84A-1-2)	RKQQC (99KS76A)	QFCSC (06ND76C)	QTHJC (75ND717C)	MCCFC (59KS19)
Lakin	4	3	4	4	4	4	–	4
Sr22TB	2–	2–	2	2	2–	;2–§	2	2–
2174	4	4	4	4	3/13–	4	4	;2–
U5615-98-120‡	2	2–/4¶	2/4	2	2–/3	2–;	2/4	2–;
U5615-98-136	2–	2–	2	2	;12–	2–;	2	;2–
U5615-98-144‡	2	2/4	2/4	2/4	;1/3+	;2–/4	2/4	2–/4
U5615-98-48	2–/4	2–/4	2	2/4	2–	2–;/4	2/4	;2–
U5616-20-154-7	2–	2–	2	2	2–	2–;	2	2–
U5616-20-47-13	2	2–	2	2	2	;2–	2	2–
U5616-20-47-10	2–	2–	2	2	2	;2–	2	2–
U5616-20-9-13	2–	2	2	2	2	2–;	2	2–
U5616-20-9-15	2–	2	2	2	2	2–;	2	;2–

†Race isolates given in parentheses.

‡Line is heterozygous at marker loci linked to *Sr22*.

§Two or more infection types present on the same plant, with the predominant type given first.

¶Two scores separated by a slash indicates heterogeneity in infection type, with the predominant phenotype given first.

both populations markers flanking *Sr22* were identified. In U5615-$F_{2:3}$, *Sr22* was flanked proximally by *Xcfa2123* at a distance of 0.6 cM and distally at 0.4 cM by cosegregating loci *Xwmc633*, *Xgwm332*, and *Xwmc790*. In the U5616-$F_{2:3}$ population, proximal marker locus *Xcfa2123* was 4.2 cM from *Sr22* and the cosegregating loci *Xwmc633*, *Xgwm332*, and *Xwmc790* were 1.8 cM distal.

Nullisomic and ditelsomic analysis of SSR loci placed *Xbarc121*, *Xcfa2019*, *Xcfa2123*, *Xgwm332*, *Xpsp3094*, *Xwmc607*, *Xwmc633*, and *Xwmc790* on the long arm of chromosome 7A and *Xgwm260* on the short arm. Marker *Xwmc607* was located on the proximal region between the centromere and the 7AL-1 breakpoint (Fig. 1a). The *Xbarc121* locus is located between deletion breakpoints 7AL-10 (fraction length [FL] = 0.49) and 7AL- 21 (FL = 0.74) and the *Xcfa2123* locus maps in the submicroscopic region between deletion breakpoints 7AL-8 and 7AL-13, both of which have FL = 0.83. The loci distal to *Sr22*, *Xwmc633*, *Xgwm332*, *Xwmc790*, *Xpsp3094* and *Xcfa2019* are located between the breakpoints 7AL-9 (FL = 0.89) and 7AL-15 (FL = 0.99) (Fig. 1a). These data place *Sr22* in the deletion interval between the 0.83 and 0.99 breakpoints.

Six SSR loci in the linkage group with *Sr22* including *Xwmc607*, *Xbarc121*, *Xwmc633*, *Xgwm332*, *Xwmc790*, and *Xcfa2019* were evaluated and determined to be polymorphic between Steinwedel and the donor line Sr22Tb (Table 4). These data, combined with our physical mapping of the markers, confirm the result of Paull et al. (1994) that the transfer from *T. boeoticum* in Sr22Tb involves at least 89% of the long arm of chromosome 7A as well as a portion of the short arm.

Although more recombination was observed in the U5616-$F_{2:3}$ population than the U5615-$F_{2:3}$ population, a comparison of the physical location of markers and genetic distances indicate low levels of recombination in both populations, particularly in the proximal portion of the chromosome. Only 3.5 and 5.6 cM of genetic distance were observed in U5615-$F_{2:3}$ and U5616-$F_{2:3}$ populations, respectively, between markers *Xgwm260* and *Xcfa2123* that span 83% of the long arm of chromosome 7A plus a portion of the short arm. In the region distal to *Sr22*, 1.1 and 1.0 cM of genetic distance was observed between SSR markers located in the deletion interval between the breakpoints for 7AL-9 (FL = 0.89) and 7AL-15 (FL = 0.99).

Linkage Analysis of $F_{3:4}$ Recombinant Populations

To develop lines having a smaller amount of *T. boeoticum*–derived chromatin, additional populations of $F_{3:4}$ families were developed from three recombinant F_2 individuals (U5615-72, U5615-98, and U5616-20; Fig. 2) Linkage

Table 3. Segregation of *Sr22* in $F_{2:3}$ and $F_{3:4}$ populations, including number of lines (*n*), the observed and expected genotypic frequencies, and χ^2 and *P* values for fit to the 1:2:1 segregation expected for a single dominant gene.

Population	*n*	Genotype	Observed	χ^2	*P*
U5615-$F_{2:3}$	138	Sr22Sr22	35	0.49	0.78
		Sr22sr22	72		
		sr22sr22	31		
U5616- $F_{2:3}$	139	Sr22Sr22	40	2.61	0.27
		Sr22sr22	72		
		sr22sr22	27		
U5615-72-$F_{3:4}$	104	Sr22Sr22	21	1.60	0.45
		Sr22sr22	53		
		sr22sr22	30		
U5615-98-$F_{3:4}$	140	Sr22Sr22	34	0.04	0.98
		Sr22sr22	71		
		sr22sr22	35		
U5616-20-$F_{3:4}$	152	Sr22Sr22	40	4.63	0.10
		Sr22sr22	64		
		sr22sr22	48		

Table 4. Simple sequence repeat markers used for linkage analysis of *Sr22*, parent allele sizes in base pairs.

Marker	Steinwedel	Sr22Tb	2174	Lakin
Xgwm260	—†	153	163	163
Xwmc607	146	89	143	143
Xbarc121	234	195	217	217
Xcfa2123	—	234	235	241
Xwmc633	260	117	221	229
Xgwm332	184	193	195	275
Xwmc790	152	89	209	100
Xpsp3094	—	228	149	183
Xcfa2019	237	238	217	217

†Unavailable data.

analyses of the $F_{3:4}$ populations were performed for loci where the U5615-72, U5615-98, and U5616-20 individuals were heterozygous. In all three $F_{3:4}$ populations, marker orders were consistent with the orders observed in the $F_{2:3}$ populations (Fig. 1). In all populations, *Xwmc633* was closest to *Sr22* with genetic distances ranging from 1.3 to 2.1 cM distal.

The U5615-72-$F_{3:4}$ population consisted of 104 lines that segregated for *Sr22*, proximal loci *Xbarc121*, *Xcfa2123*, *Xwmc607*, and *Xgwm260* as well as distal loci *Xgwm332*, *Xpsp3094*, *Xwmc633*, and *Xwmc790*. Recombination occurred between the *Xcfa2019* locus and *Sr22* in the previous generation; thus, the U5615-72-$F_{3:4}$ population was fixed for alleles from parent line 2174 in the region of 7AL distal to the *Xcfa2019* locus (Fig. 2). In the related U5615-98 $F_{3:4}$, 140 lines segregated for *Sr22* and distal loci *Xcfa2019*, *Xgwm332*, *Xpsp3094*, *Xwmc633*, and *Xwmc790*. This population was fixed for alleles from parent line 2174 at the SSR loci proximal to *Sr22* (Fig. 2). In both cases, recombination levels were greater ($P < 0.01$) than observed in the original U5615-$F_{2:3}$ and were similar to the distances observed for the U5616-$F_{2:3}$ and U5616-20- $F_{3:4}$ populations.

Identification of Recombinants

Several recombinant genotypes were identified that carry reduced levels of *T. boeoticum* chromatin associated with *Sr22*. In the U5615-98-$F_{3:4}$ population that was fixed for

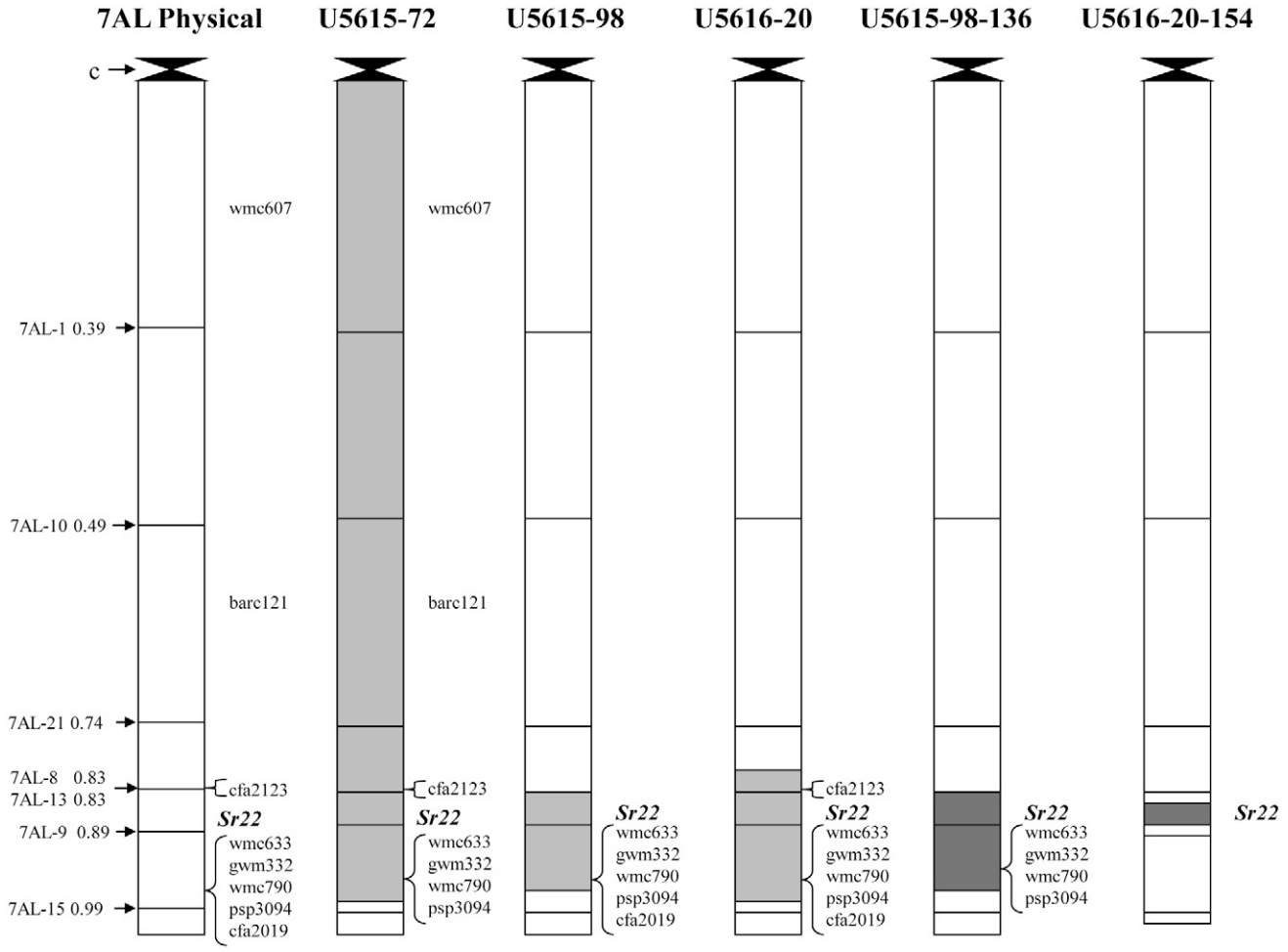

Figure 2. Physical maps of the long arm of chromosome 7A showing *Triticum boeoticum* chromatin in recombinants identified from 2174/Sr22Tb (U5615) and Lakin/Sr22Tb (U5616) $F_{2:3}$ and $F_{3:4}$ populations. Light gray shading represents regions heterozygous for the markers noted to the left of the chromosome. Regions shaded in dark gray represent regions homozygous for *T. boeoticum* chromatin. Deletion breakpoints are indicated as solid lines. Line names and fraction lengths are at left. C indicates the position of the centromere. Marker gwm260 is not shown because it is located on the short arm of chromosome 7A.

alleles from parent line 2174 at marker loci proximal to *Sr22*, seven genotypes were identified that are resistant to stem rust and in which recombination had occurred between *Sr22* and the most distal marker *Xcfa2019*. Three lines fixed for *T. boeoticum* alleles at distal loci *Xwmc633*, *Xgwm332*, *Xwmc790*, and *Xpsp3094* were homozygous resistant to race RKQQC, of which recombinant U5615-98-136 is shown as example in Fig. 2. Two of these recombinants, U5615-98-136 and U5616-98-48, were evaluated for reaction to nine additional races of stem rust, including TTKSK and TTKST, and exhibited widely effective resistance to all races (Table 2). Four recombinants were heterozygous for *Sr22*, *Xwmc633*, *Xgwm332*, *Xwmc790*, and *Xpsp3094*, and homozygous for alleles from parent line 2174 at *Xcfa2019*. Two of these lines (U5615-98-120 and U5615-98-144) were tested and segregated for resistance to diverse races of stem rust (Table 2). These populations can be used to identify new recombinants with further reduced alien chromatin in subsequent generations of inbreeding.

In the U5616-20 population, three genotypes were identified as segregating for resistance to stem rust race RKQQC and homozygous for Lakin alleles at flanking markers *Xcfa2123* and *Xwmc633*, of which recombinant U5616-20-154 is shown as an example in Fig. 2. Plants were self-pollinated and five lines homozygous resistant to all tested races of stem rust were identified (Table 2).

CONCLUSION

Although the stem rust resistance gene *Sr22* provides resistance to all known races of *Pgt*, it has not been widely used in agriculture. This is likely due to detrimental effects associated with introgression from the A^m genome diploid donor species, *T. boeoticum*. Our data support the hypothesis of Paull et al. (1994) that the proximal regions of both the short and long arm of chromosome 7A are comprised of *T. boeoticum* chromatin in lines having *Sr22* derived from Sr22Tb. Paull et al. (1994) reported that the restriction fragment length polymorphism locus polymorphic between *T. boeoticum* and hexaploid wheat in the distal region of 7AL, *Xpsr119*, was not transmitted from *T. boeoticum*. They speculated that the terminal region of 7AL in *Sr22* hexaploid lines is likely of *T. aestivum* origin while the proximal region was derived from *T. boeoticum*. The 13 SSR loci mapped in this study segregated into two unlinked groups, although all markers except *Xgwm260* were located on the long arm of 7A. Four of the SSR loci that are located in the most distal region of 7AL were not linked to *Sr22* and underwent much greater levels of recombination than observed in the *Sr22* region (data not shown). These data agree with the hypothesis that the terminal region of the long arm of 7A in the Sr22Tb donor parent is of *T. aestivum* origin.

Low levels of recombination were observed in the $F_{2:3}$ populations when compared with previous intraspecific SSR maps of chromosome 7A. Somers et al. (2004) reported the distance between *Xgwm260* and *Xcfa2019* to be 37 cM. The same interval in our study represented 5.6 cM in the U5615-$F_{2:3}$ population and 12.6 cM in the U5616-$F_{2:3}$ population. Although both populations exhibited suppressed recombination, greater recombination was observed in the $F_{2:3}$ population from the cross Lakin/Sr22Tb than the 2174/Sr22Tb. However, similar levels of recombination in the region were observed in the $F_{3:4}$ populations from both crosses, indicating that the observed reduction in the original 2174/Sr22Tb $F_{2:3}$ may have been specific to that population.

The and McIntosh (1975) reported reduced transmission of *Sr22* in pooled populations of lines with *Sr22* from both *T. monococcum* and *T. boeoticum*. In this study, segregation for 7AL alleles including *Sr22* did not deviate significantly from the expected 1:2:1 ratio in all populations.

Previously, the SSR loci *Xcfa2123* and *Xcfa2019* were identified as flanking markers for *Sr22* (Khan et al., 2005). These same markers were polymorphic in both of our populations and were found to flank *Sr22*. Reduced levels of recombination associated with *Sr22* were identified by Khan et al. (2005) in a single F_4–derived, single seed descent population, on which mapping was done only on homozygous resistant and susceptible lines. In the present study, all individuals in multiple populations were genotyped with codominant markers allowing for more accurate estimates of genetic distances. Mapping in the $F_{2:3}$ allowed for the identification of critical recombinants with reduced *T. boeoticum* chromatin. From these recombinants, additional mapping populations were made and recombinants with even further reduced alien chromatin were identified.

The recombinant lines carrying reduced *T. boeoticum* chromatin identified in this study will be useful in breeding programs. Using these lines as donor parents should help to meliorate the negative effects associated with *Sr22*. These recombinant lines are being backcrossed to hard and soft winter wheat cultivars to produce populations and NILs for which the effects of the introgression on agronomic performance can be evaluated. The marker loci documented in this study will prove useful in the marker-assisted selection of *Sr22*. The most closely linked marker locus to *Sr22* across all populations was the distal locus *Xwmc633*, while the proximal flanking marker *Xcfa2123* could also be used but greater recombination between this locus and *Sr22* was observed. Markers identified in this study can be used for selection among the progeny of the recombinant lines from the U5615-98 population to further reduce the size of the *T. boeoticum* chromosome segment. Homozygous resistant $F_{4:5}$ progeny have been identified from each of the U5616-20–derived recombinant plants. The use of marker-assisted selection in progeny from crosses with the U5616-20 recombinants will require the development of markers more closely linked to *Sr22*. Recombinants with reduced *T. boeoticum* chromatin are available on request for development of wheat cultivars having resistance to stem rust.

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